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# AGRICULTURAL AND BIOLOGICAL CHEMISTRY

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## Note

Aroma Components of Fresh  
Sugar Cane JuiceYukiko TOKITOMO, Akio KOBAYASHI\*  
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In previous papers,<sup>1-3)</sup> we have reported the compounds responsible for the sugary flavor of raw cane sugar. Many aroma components have been identified from cane molasses, and the most important key compound of the sugary flavor was identified to be 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon).<sup>3)</sup> 4-Hydroxy-3-methoxybenzaldehyde (vanillin), 3-hydroxy-2-methyl-4-pyranone, 2-hydroxy-3-methyl-2-cyclopentenone, 4-nonanolide, 4-vinylphenol, and 2,6-dimethoxyphenol were also thought to contribute to the sugary flavor. These aroma components seemed to be a mixture of naturally occurring products of sugar cane and by-products arising during raw cane sugar manufacturing with a high temperature (105°C) and alkaline conditions. We investigated the aroma components of fresh juice from sugar cane and compared them with those of cane molasses to estimate

the formation of the sugary flavor. For extraction, fractionation, and identification of aroma components of cane juice we followed the same procedures as for cane molasses.<sup>1,3)</sup>

The cane juice was produced from cane (*Saccharum officinarum* var. NCO 376) in Nago, Okinawa, harvested in January, 1979. Without heating and adding lime, the juice was already dark green and had a characteristic sweet aroma like that of brown sugar with a green note.

The juice (11.6 kg) was extracted with ether continuously using liquid-liquid extracting apparatus for 40 hr, and 5.42 g (0.047%) of aroma concentrate with a green and sugary odor was obtained. The aroma concentrate (4.0 g) was separated by silica-gel column chromatography as shown in Table I. The most characteristic aroma existed in fraction 11 (Fr. 11), which was further separated by preparative gas chromatography using a Shimadzu GC-4A gas chromatograph equipped with a thermal conductivity detector. By sniffing the GC effluents, smoky and slightly sugary aromas were found concentrated between retention times of 53 and 65 min (column, 10% SP2100 on 100/120 Chromosorb AW-DMCS; glass packed column 3 m × 4 mm i.d.; column temp., programmed from 70 to 180°C, at a rate of 2°C/min). This fraction was trapped (Fr. 11-trap) and was rechromatographed with a Shimadzu GC-6A gas chromatograph equipped with a flame ionization detector and glass SCOT column as described in Fig. 1. Identification of the individual peaks was performed by matching of their mass spectra and their GC retention times with those of authentic samples. GC-MS spectra were recorded with a Hitachi RMU-6MG mass spectrometer combined with a Hitachi 063 gas chromato-

TABLE I. FRACTIONATION OF THE AROMA CONCENTRATE (4.0 g) BY SILICA-GEL  
COLUMN\* CHROMATOGRAPHY

| Fraction | Solvent                        |    |     | Volume<br>(ml) | Yield<br>(g) | Aroma             |
|----------|--------------------------------|----|-----|----------------|--------------|-------------------|
|          | Benzene-Ethyl acetate-Methanol |    |     |                |              |                   |
| 1        | 100                            |    |     | 100            | 0            | Inodorous         |
| 2        | 100                            |    |     | 100            | 0.57         | Rubber-like       |
| 3        | 100                            | :  | 1   | 100            | 0.46         | Fruity            |
| 4        | 50                             | :  | 1   | 100            | 0.51         | Phenolic          |
| 5        | 25                             | :  | 1   | 60             | 0.13         | Inodorous         |
| 6        | 15                             | :  | 1   | 60             | 0.06         | Medicinal         |
| 7        | 8                              | :  | 1   | 80             | 0.17         | Medicinal, floral |
| 8        | 4                              | :  | 1   | 60             | 0.23         | Phenolic          |
| 9        | 4                              | :  | 1   | 75             | 0.23         | Sweet, green      |
| 10       | 2                              | :  | 1   | 75             | 0.35         | Green, sugary     |
| 11       | 1                              | :  | 1   | 80             | 0.18         | Sweet, sugary     |
| 12       |                                |    | 100 | 80             | 0.17         | Acidic, green     |
| 13       |                                |    | 100 | 110            | 0.32         | Acidic, sweet     |
| 14       |                                | 50 | :   | 1              | 60           | Inodorous         |
| 15       |                                | 10 | :   | 1              | 50           |                   |
| 16       |                                | 1  | :   | 1              | 50           |                   |

\* Column: 2.4 cm (i.d.) × 56 cm, Wakogel C-200, 70 g.

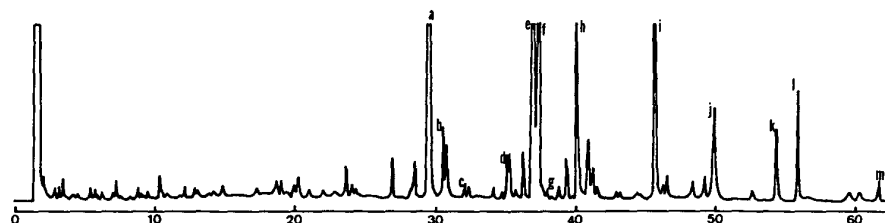


FIG. 1. Gas Chromatogram of Fr. 11-Trap.

Analytical conditions: Column, 30 m  $\times$  0.28 mm i.d. glass SCOT column coated with FFAP; column temp., programmed from 70°C to 180°C at a rate of 2°C/min; temp. of injector and detector, 190°C; carrier gas, nitrogen; flow rate of 1.2 ml/min at column inlet.

Identified peaks: a), hexanoic acid and 2-methoxyphenol; b), benzyl alcohol; c), 2-phenylethanol; d), heptanoic acid; e) phenol; f), 4-nonanolide; g), 3-phenyl-1-propanol; h) nonanoic acid; i), 4-hydroxy-3-methoxystyrene; j), 3-phenyl-2-propenol; k), 4-vinylphenol; l), phthalate (contaminant); m), 4-hydroxy-2-methoxybenzaldehyde.

graph. The column was a 30 m  $\times$  0.28 mm i.d. glass SCOT column coated with Thermo 600T and the oven temperature was programmed from 70 to 220°C at a rate of 3°C/min. The ionization energy was 20 eV.

As shown in Fig. 1, the main components of the Fr. 11-trap, a), b), c), e), f), i), k), and m) were also identified in cane molasses, among which 4-nonanolide, 4-vinylphenol, and 4-hydroxy-3-methoxybenzaldehyde were considered to contribute to the sugary aroma of this fraction. Therefore, these phenolic compounds and lactone originate from raw cane juice and contribute to the sugary aroma.

On the other hand, the other sugary flavor components, 3-hydroxy-2-methyl-4-pyranone<sup>3,4)</sup> and 2-hydroxy-3-methyl-2-cyclopentenone,<sup>1)</sup> could not be found in fresh cane juice and these typical volatile products were assumed to be formed from sugar by heating.<sup>5)</sup>

Sotolon could not be identified in cane juice. Two of the authors claimed in another paper<sup>6)</sup> that sotolon was prepared by condensation of pyruvate and 2-oxobutyrate, 2-oxoglutarate, or glutamate which exist in cane juice under the same conditions as those used in raw cane sugar manufacturing. The present result also supports the formation of this key compound of the sugary flavor during sugar processing, but the presence of sotolon in fresh cane juice is still undeniable because the odor threshold value of sotolon is very low (0.01 ppb in water) and the characteristic aroma of Fr. 11-trap could not be regenerated by mixing the identified compounds. The identification of

minor components of this aroma are now in progress using a large quantity of fresh cane juice with more sophisticated techniques.

**Acknowledgments.** We wish to thank Dr. Y. Nakasone of Ryukyu University and the Hokubu Sugar Corporation in Nago for kindly supplying the cane juice. We are also indebted to Dr. S. Muraki, Takasago Perfumery Co., Ltd., for obtaining the mass spectra by capillary GC-MS. We express special appreciation to the late Professor S. Takei for his valuable advice and encouragement.

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## Note

### Effects of a Selenium L-Lysine on *Candida pelli*

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Selenium, a homologue of sulfur, is an essential micronutrient for many bacteria. The biological role of selenium has received considerable attention in amino- $\beta$ -hydroxypropionylthionine ( $\alpha$ -amino- $\gamma$ -methylseleno- $\beta$ -thionine) in polypeptide chain containing enzymes.<sup>1)</sup> Se-( $\beta$ -amino- $\gamma$ -methylseleno- $\beta$ -thionine), in which the  $\gamma$ -n is replaced by a selenium atom, has been reported as a competitive inhibitor of lysine

FIG. 1. Effect of L-Seleno-lysine on the growth of *C. pelli*. A 0.1 ml aliquot of the see various concentrations of L-Seleno-lysine at 28°C on a reciprocating sh relationship between the c

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